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Effect of nicotine replacement and quitting smoking on circulating adhesion molecule profiles (sICAM-1, sCD44v5, sCD44v6)

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Abstract

Background Soluble ICAM-1 (sICAM-1; sCD54), sCD44v5 and sCD44v6 are circulating adhesion molecules, with immunomodulatory potential, that have been frequently attributed diagnostic, prognostic and aetiological significance in a number of inflammatory and malignant diseases. We have previously shown that systemic concentrations of these molecules are increased significantly in tobacco smokers, but reduce to within normal levels at 12 months following successful quitting.

Materials and methods We have been able to extend these observations by measuring levels before and 4, 8, 22 and 52 weeks after smoking cessation in subjects receiving high-dose nicotine replacement therapy (25 mg of nicotine; $n = 34$) or placebo patches ($n = 34$) for 26 weeks. Smoking cessation was confirmed by regular measurement of expired-air CO levels and by plasma cotinine analysis.

Results Plasma sICAM-1, sCD44v5 and sCD44v6 concentrations all declined rapidly within 4 weeks of smoking cessation ($P < 0.001$ for all declines). Additionally, no differences were observed between those using nicotine replacement and those who were not for sICAM-1, sCD44v5, or sCD44v6.

Conclusions The recovery in smoking-associated adhesion molecule profiles represents an almost immediate beneficial effect of smoking cessation. Nicotine replacement therapy is an effective aid to quitting and does not affect these recoveries. The elevated levels of these important risk factors in smokers (sICAM-1, sCD44v5 and sCD44v6) are linked to noxious element(s) in tobacco smoke other than nicotine or nicotine metabolites.

Keywords Adhesion molecules, CD44, ICAM-1, nicotine replacement therapy, smoking, tobacco.
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Introduction

Tobacco smoking represents a major risk factor for many distinct disease entities, including several cancers, chronic obstructive pulmonary disease, chronic inflammatory periodontal disease, osteoporosis, and atherosclerosis and its clinical sequelae. Indeed, the World Health Organization has estimated that the number of deaths attributable to tobacco-induced disease will reach 100 million over the first two decades of this century [1]. Therefore, tobacco smoking represents an enormous health problem.

Tobacco smoke contains large numbers of potent carcinogens and toxins which exert profound effects on several components of the immune system [2–7]. Lymphocyte circulation and the recruitment of leukocytes from the vascular system to sites of infection/inflammation lie at the heart of the immune response. This leukocyte trafficking is controlled by a series of interactions between several adhesion molecules and their ligands [6,8–10]. Recent evidence has shown that smoking leads to altered cell-bound adhesion molecule expression profiles in several diseased tissues [6,11,12] and to increased shedding of soluble forms of specific adhesion

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molecules, most notably soluble intercellular adhesion molecule-1 (sICAM-1) [5,6,13] and variant, tumour-associated sCD44 isoforms (sCD44 containing the product of exon 5, and sCD44 containing the product of exon 6) [14,15].

The biological functions of most soluble adhesion molecules have yet to be definitively ascertained. However, there is increasing evidence that sICAM-1 and sCD44 molecules remain bioactive and are immunomodulatory, as reviewed recently [6]. Additionally, there is an extensive literature attributing diagnostic and prognostic significance to altered circulating profiles of sICAM-1, sCD44v5 and sCD44v6 in inflammatory and malignant diseases [6,13,16–27].

Previously, we have shown that tobacco smoking leads to a dose-dependent increase in the concentration of circulating isoforms of sICAM-1 [5], sCD44v5 and sCD44v6 [15], and that long-term (1 year) cessation of tobacco use results in a significant reduction in the systemic load of these specific adhesion molecules [5,15]. This implies that tobacco smoke directly influences the production and release of sICAM-1, sCD44v5 and sCD44v6. However, the time period over which recovery occurs following quitting, and the components of cigarette smoke that lead to increased soluble adhesion molecule levels, are unknown. Therefore, we performed an analysis of sICAM-1, sCD44v5 and sCD44v6 profiles in plasma samples obtained from subjects who stopped smoking, with or without exposure to high doses of nicotine delivered by a transdermal nicotine patch.

Subjects, materials and methods

Subject inclusion criteria

All subjects had taken part in the Collaborative European Anti-Smoking Evaluation (CEASE) trial [28]. This was a large placebo-controlled trial ($n = 3560$) of nicotine replacement therapy (NRT) evaluating five levels of dose and duration of the 16-h (daytime) transdermal nicotine patch (Pharmacia and Upjohn, Sweden), and performed under the auspices of the European Respiratory Society. Successful quitters were selected retrospectively from two of the trial treatment arms: those who used high-dose (25 mg) nicotine patches for 26 weeks ($n = 34$), and those who were assigned placebo patches ($n = 34$). In the original trial, 110 (25 mg) and 71 (placebo) participants reported continuous abstinence from week 2 to week 52, verified by expired-air carbon monoxide levels < 10 p.p.m. (portable CO monitor, Bedfont Scientific, Rochester, Kent, UK) determined at 2, 4, 8, 12, 22, 26 and 52 weeks.

We used additional inclusion criteria when selecting cases for the present study in order to provide the strongest assurance that: (a) participants had not stopped smoking before treatment started (baseline); (b) participants were abstinent from smoking throughout the year; (c) participants assigned to the nicotine patch received substantial doses until week 22. Hence, we additionally required that baseline plasma cotinine levels (determined by gas-liquid chromatography) exceeded 50 ng mL^{-1} for all participants, that plasma cotinine levels were < 15 ng mL^{-1} at 4, 8, 22 and 52 weeks in the placebo group and < 15 ng mL^{-1} at 52 weeks in the nicotine group, and that plasma cotinine levels exceeded 30% of baseline smoking levels at 4, 8 and 22 weeks in the nicotine group. The decision to use a minimum 30% replacement level was largely arbitrary, being approximately half the mean level in the trial [28]. These inclusion criteria were set in advance. Applying these criteria reduced the number of eligible cases to 34 and 35 in the placebo and nicotine groups, respectively. The baseline plasma sample for one nicotine subject was found to be contaminated on analysis for adhesion molecule concentration, leaving final study samples of 34 participants in each group. Ethical approval for the study was obtained from the local Ethics Committee at Guy's and St Thomas's Hospital, London.

Analysis of circulating sICAM, sCD44v5 and sCD44v6 concentrations

Plasma samples taken at each visit (baseline, 4, 8, 22 and 52 weeks) were assayed for sICAM, sCD44v5 and sCD44v6 levels by domain-specific ELISA. The mean intra-assay coefficient of variation (%) is reported to be 4·4, 3·6 and 3·0 for the sICAM-1 (R & D Systems), sCD44v5 (Bender MedSystems) and sCD44v6 (Bender MedSystems) ELISAs, respectively. The mean interassay coefficient of variation (%) has been determined to be 7·4, 5·8 and 4·2 for the sICAM-1, sCD44v5 and sCD44v6 ELISAs, respectively. Each ELISA assay has a sensitivity of < 0·4 ng mL^{-1}. All adhesion molecule assays were performed in duplicate and blind to NRT status.

Statistical analyses

The *t*-test and chi-square tests were used to examine significant differences between the NRT and placebo groups with respect to age, gender (chi-square), baseline tobacco exposure, and baseline concentrations of sICAM-1, sCD44v5 and sCD44v6. Simple within- and between-subject analyses of variance models were used to test for differences in circulating adhesion molecule concentrations over time.

Results

There were no significant differences between the NRT and placebo groups with respect to age (mean 45 and 44 year, respectively), gender (19 and 14 females, respectively) or baseline tobacco consumption (mean 25 and 23 cigarettes day^{-1}; mean 252 and 238 ng mL^{-1} plasma cotinine, respectively). Expired air carbon monoxide levels confirmed their quit smoking status throughout the year (Fig. 1). The active patch produced nicotine and cotinine levels throughout the treatment similar to baseline smoking levels (Figs 2 and 3).

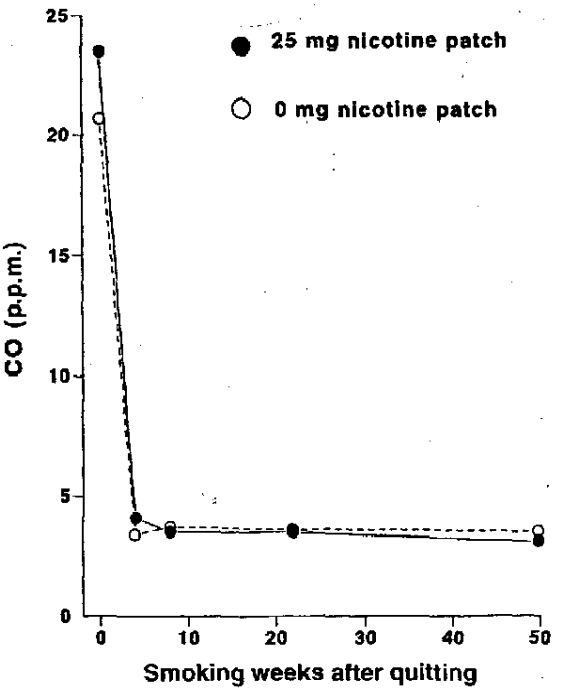

Figure 1 Expired-air CO concentrations over 52 weeks in the nicotine replacement therapy and placebo groups. Mean CO concentrations are given. Error bars represent the standard error.

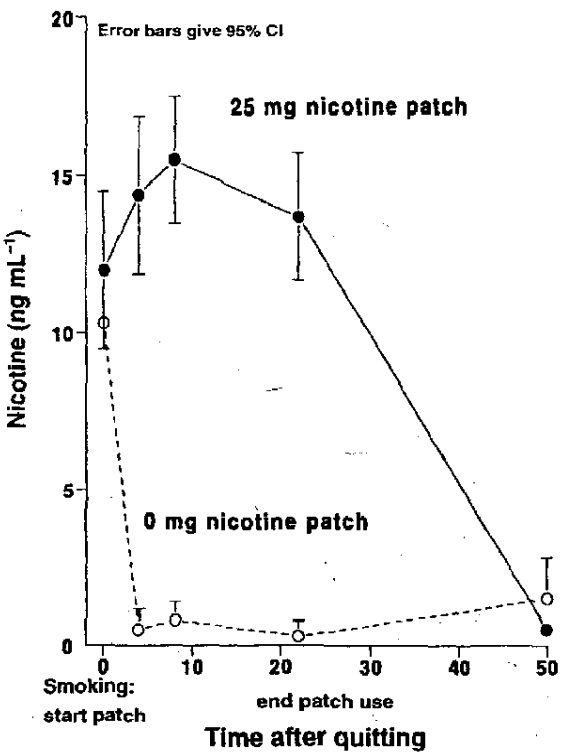

Figure 2 Plasma nicotine concentrations over 52 weeks in the nicotine replacement therapy and placebo groups. Mean nicotine concentrations are given. Error bars represent the standard error.

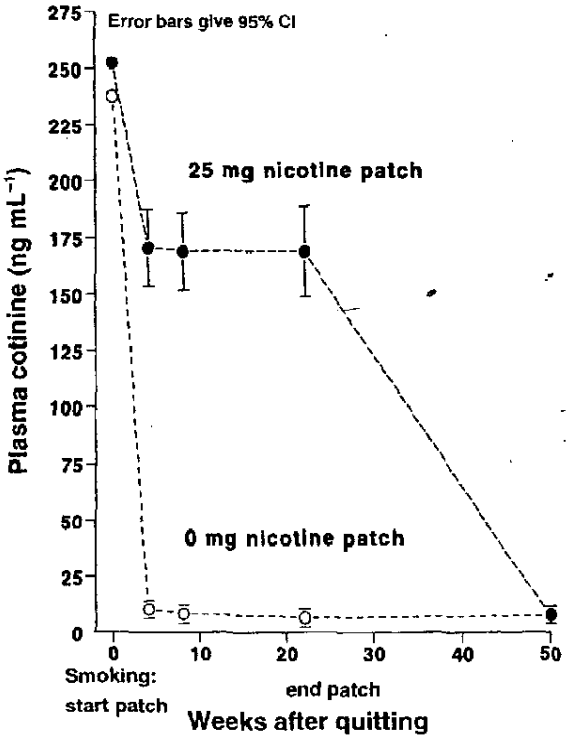

Figure 3 Plasma cotinine concentrations over 52 weeks in the nicotine replacement therapy and placebo groups. Mean cotinine concentrations are given. Error bars represent the standard error.

Soluble adhesion molecule profiles

The baseline plasma concentrations of sICAM-1, sCD44v5 and sCD44v6 (presented in Figs 4, 5 and 6, respectively) were similar to those previously reported for smokers [5,6,14,15]. There were no significant differences between plasma levels of sICAM-1, sCD44v5 or sCD44v6 in the NRT or placebo groups at baseline.

On stopping smoking, the decline in the circulating concentration of each adhesion molecule was rapid, with the majority of the change occurring in the first 4 weeks, regardless of whether the subject received active NRT or placebo (see Figs 4, 5 and 6; $P < 0{\cdot}001$ for all declines).

There was no evidence of a difference in circulating concentration throughout the year between those taking nicotine and those on placebo ($P > 0{\cdot}05$ for all comparisons).

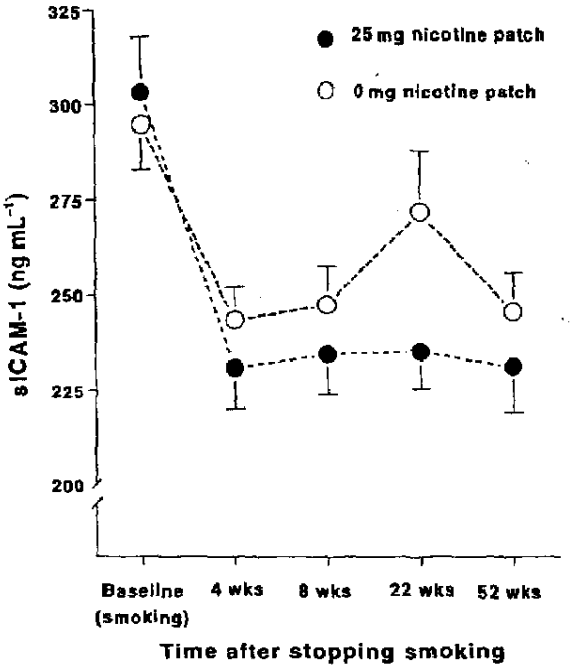

Figure 4 Effect of stopping smoking on circulating concentrations of sICAM-1. Mean sICAM-1 concentrations are given. Error bars represent the standard error.

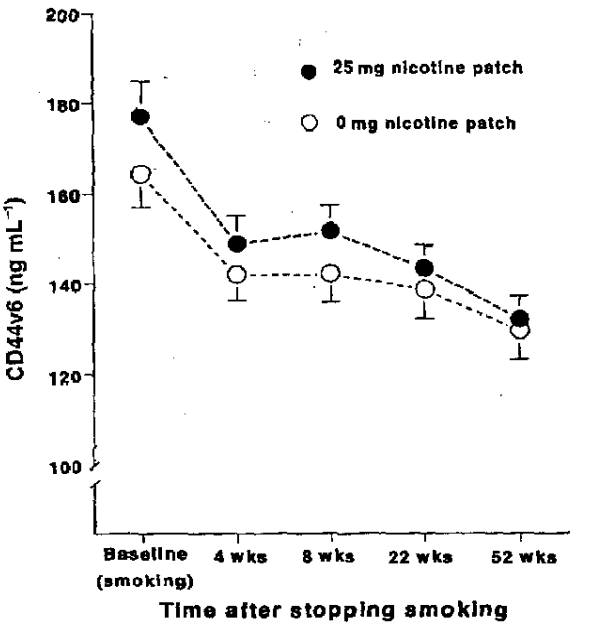

Figure 6 Effect of stopping smoking on circulating concentrations of sCD44v6. Mean CD44v6 concentrations are given. Error bars represent the standard error.

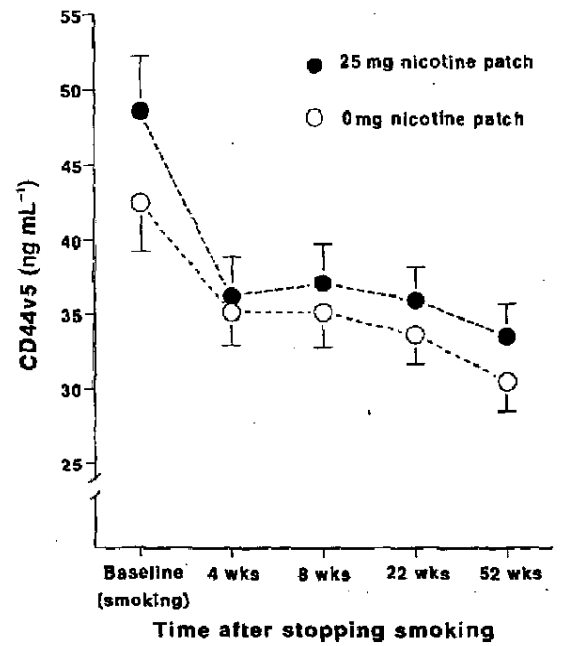

Figure 5 Effect of stopping smoking on circulating concentrations of sCD44v5. Mean CD44v5 concentrations are given. Error bars represent the standard error.

Discussion

There was a significant and rapid decline in the circulating concentration of each adhesion molecule under investigation on stopping tobacco smoking. It is interesting to note that in the placebo group, levels of sICAM-1 showed some increase at week 22 before declining again thereafter. We have no explanation for the rise in mean sICAM-1 concentration at this point, as plasma cotinine, plasma nicotine and expired-air CO levels did not indicate any lapses to smoking. Plasma CD44v5 and CD44v6 concentrations, which dropped markedly at week 4, showed a tendency for a gradual decline thereafter. These declines are in sharp contrast to the high, stable concentrations seen in a random sample of trial participants who continued to smoke over the same period [5,15].

As NRT did not influence the rapid decline in soluble adhesion molecule concentrations, this study provides strong *in vivo* evidence that it is a constituent(s) of tobacco smoke other than nicotine, or its metabolites, that is responsible for the elevated sICAM, sCD44v5 and sCD44v6 concentrations noted in habitual smokers.

More importantly, effective smoking cessation will result in a rapid decline in the systemic load of sICAM, sCD44v5 and sCD44v6, which could have important, and direct, medical benefits. sICAM-1, for example, has been reported to stimulate proteloytic enzyme release from neutrophils as a consequence of interaction with β_2-integrin ligand molecules on the neutrophil surface [23], and, thus, may be expected to contribute to tissue degradation in several smoking-induced disease entities. Studies have also shown

that sICAM-1 may possess several other immunodulatory functions, including the induction of pro-inflammatory cytokine production by macrophages [27], promotion of angiogenesis [29], competitive inhibition of leukocyte–endothelial interactions [25], and the inhibition of immune surveillance [24]. While there is a pressing need for research into the biological significance of sCD44 molecules, there are several reports that sCD44 molecules may also be bioactive and immunomodulatory. For example, specific sCD44 molecules can bind the major matrix components, hyaluronate and fibronectin [26], inhibit human peripheral blood lymphocyte binding to endothelial cells [26], and can influence the metastatic potential of tumour cells [6,26,30,31].

Inhaled nicotine is responsible for maintaining tobacco addiction. Nicotine replacement therapy, however, is highly effective in reducing symptoms of withdrawal from tobacco use, can double the chance of successful long-term cessation and is a safe medication with few side-effects [32]. Overall, subjects in the CEASE trial who were on 25-mg/16-h nicotine patches were more successful in quitting smoking than those on placebo ($P < 0{\cdot}001$) [28]. We have shown that the recovery in smoking-associated adhesion molecule profiles is not compromised by prolonged periods of using high-dose transdermal nicotine replacement.

In summary, smoking cessation results in a dramatic and rapid decline in the circulating concentrations of the immunomodulatory molecules sICAM, sCD44v5 and sCD44v6. In addition, transdermal nicotine, which represents a valuable aid to quitting, does not affect this recovery.

References

1 World Health Organization. Fact Sheets 221 (http://www.who.International/inf-fs/en/fact221.html) and 222 (http://www.who.int/inf-fs/en/fact222.html).

2 Barbour SE, Nakashima K, Zhang JB, Tangada S, Hahn CL, Schenkein HA *et al.* Tobacco and smoking: environmental factors that effect the host response and have an impact on peridontal health. *Crit Rev Oral Biol Med* 1997;8:437–60.

3 Gunsolley JC, Pandey JP, Quinn SM, Tew J, Schenkein HA. The effect of race, smoking and immunoglobulin allotypes on IgG subclass concentrations. *J Periodontal Res* 1997;32:381–7.

4 Pitzer JE, Del Zoppo GJ, Schmid-Schönbein GW. Neutrophil activation in smokers. *Biorheology* 1996;33:45–58.

5 Scott DA, Stapleton JA, Coward PY, Wilson RF, Sutherland G *et al.* Dramatic decline in circulating intercellular adhesion molecule-1 concentration on quitting tobacco smoking. *Blood Cells Mol Dis* 2000;26:255–8.

6 Scott DA, Palmer RM. The influence of tobacco smoking on adhesion molecule profiles. *Tobacco Induced Dis* 2002;1:8–25.

7 Sopori ML, Kozak W. Immunomodulatory effects of cigarette smoke. *J Neuroimmunol* 1998;83:148–56.

8 Hellewell PG. Adhesion molecule strategies. *Pulm Pharmacol Therapeut* 1999;12:137–41.

9 Johnson P, Maiti A, Brown KL, Li R. A role for the cell adhesion molecule CD44 and sulfation in leukocyte-endothelial cell adhesion during an inflammatory response? *Biochem Pharmacol* 2000;59:455–65.

10 Steeber DA, Tedder TF. Adhesion molecule cascades direct lymphocyte recirculation and leukocyte migration during inflammation. *Immunologic Res* 2001;22:299–317.

11 Rezavandi K, Palmer RM, Odell EW, Scott DA, Wilson RF. Expression of E-Selectin and ICAM-1 in gingival tissues of smokers and non-smokers with periodontitis. *J Oral Pathol Med* 2002;31:59–64.

12 Schaberg T, Rau M, Oerter R, Liebers U, Rahn W *et al.* Expression of adhesion molecules in peripheral pulmonary vessels from smokers and nonsmokers. *Lung* 1996;174:71–81.

13 Grothey A, Heistermann P, Philippou S, Voigtmann R. Serum levels of soluble intercellular adhesion molecule-1 (ICAM-1, CD54) in patients with non-small-cell lung cancer. correlation with histological expression of ICAM-1 and tumour stage. *Br J Cancer* 1998;77:801–7.

14 Kittl EM, Ruckser R, Selleny S, Samek V, Hofmann J *et al.* Evaluation of soluble CD44 splice variant v5 in the diagnosis and follow-up in breast cancer patients. *Exp Clin Immunogenet* 1997;14:264–72.

15 Scott DA, Stapleton JA, Palmer RM, Wilson RF, Sutherland G *et al.* Plasma concentrations of reputed tumor-associated soluble CD44 isoforms (v5 and v6) in smokers are dose related and decline on smoking cessation. *Cancer Epidemiol Biomarkers Prev* 2000;9:1211–4.

16 Banks RE, Gearing AJH, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) in human malignancies. *Br J Cancer* 1993;68:122–4.

17 Sasaki K, Niitsu N. Elevated serum levels of soluble CD44 variant 6 are correlated with shorter survival in aggressive non-Hodgkin's lymphoma. *Eur J Haematol* 2000;65:195–202.

18 Yamane N, Tsujitani S, Makino M, Maeta M, Kaibara N. Soluble CD44 variant 6 as a prognostic indicator in patients with colorectal cancer. *Oncology* 1999;56:232–8.

19 Haberhauer G, Kittl EM, Skoumal M, Hubl W, Wagner E *et al.* Increased serum levels of soluble CD44-isoform v5 in rheumatic diseases are restricted to seropositive rheumatoid arthritis. *Acta Med Austriaca* 1997;24:23–5.

20 O'Malley T, Ludlam CA, Riemermsa RA, Fox KAA. Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1). Potential risk factor for the acute coronary syndromes. *Eur Heart J* 2001;22:1226–34.

21 Rohde LE, Lee RT, Rivero J, Jamacochian M, Arroyo LH *et al.* Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 1998;18:1765–70.

22 Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule-1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 1998;351:88–92.

23 Barnett CC, Moore EE, Moore FA, Biffl WL, Partrick DA. Soluble intercellullar adhesion molecule-1 provokes polymorphonuclear leukocyte elastase release by CD18. *Surgery* 1996;120:395–402.

24 Becker JC, Termeer C, Schmidt RE, Brocker EB. Soluble intercellular adhesion molecule-1 inhibits MHC-restricted specific T cell/tumor interaction. *J Immunol* 1993;151:7224–32.

25 Rieckmann P, Michel U, Albrecht M, Bruck W, Wockel L, Felgenhauer K. Soluble forms of intercellular adhesion molecule-1 (ICAM-1) block lymphocyte attachment to

cerebral endothelial cells. *J Neuroimmunol* 1995;60:9–15.
26 Ristamaki R, Joensuu H, Gron-Virta K, Salmi M, Jalkanen S. Origin and function of circulating CD44 in non-Hodgkin's lymphoma. *J Immunol* 1997;158:3000–8.
27 Schmal H, Czermak BJ, Lentsch AB, Bless NM, Beck-Schimmer B *et al.* Soluble ICAM-1 activates lung macrophages and enhances lung injury. *J Immunol* 1998;161:3685–93.
28 Tonnesen P, Paoletti P, Gustavsson G, Russell MA, Saracci R *et al.* Higher dosage nicotine patches increase one-year smoking cessation rates: results from the European CEASE trial. *Eur Respir J* 1999;13:238–46.
29 Gho YS, Kleinman HK, Sosne G. Angiogenic activity of human soluble intercellular adhesion molecule-1. *Cancer Res* 1999;59:5128–32.
30 Yu Q, Stamenkovic I. Localization of matrix metallproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev* 1999;13:35–48.
31 Peterson RM, Yu Q, Stamenkovic I, Toole BP. Perturbation of hyaluronan interactions by soluble CD44 inhibits growth of murine mammary carcinoma cells in ascites. *Am J Pathol* 2000;156:2159–67.
32 Fiore MC, Bailey WC, Cohen SJ, Dorfman SF, Goldstein MG *et al. Treating Tobacco Use and Dependence; Clinical Practice Guideline.* Rockville, MD: US Department of Health and Human Sciences, Public Health Service;2000 (available at http://www.surgeongeneral.gov/tobacco/).